A SIMPLE METHOD FOR ISOLATING ANAEROBES IN PURE CULTURE.*

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The difficulty of isolating anaerobes in pure culture has always been a serious handicap to the study of these organisms. The following method has proved so simple and easy of application that its publication seemed warranted.

Sixteen-ounce, French square, wide-mouthed bottles are plugged with cotton and sterilized by dry heat. With the bottles lying on their sides, sufficient blood agar is poured in to form a layer 2 to 5 mm. in thickness, and allowed to harden. As soon as the agar has hardened, the bottles should be turned on the opposite side, thus bringing the medium uppermost and preventing the water of condensation from running down on it.

If sterile blood for blood agar plates is not available Dorset's egg medium to which 1 per cent of dextrose has been added may be used. The medium is run into the bottles which must be kept lying on their sides, coagulated, and sterilized in the same way as Dorset's medium. By bringing up the temperature very slowly, the sterilization may all be done at once by heating in the autoclave for 15 minutes under 10 pounds' pressure. As the colonies are difficult to see on this yellowish-white medium, this difficulty should be obviated by the addition of litmus to the medium before sterilization. Under the anaerobic conditions produced in the bottle after inoculation, the litmus is more or less completely bleached. After the readmission of air, however, the color soon returns and assists very materially, not only by rendering the colonies more easily visible, but also by differentiating acid producers.

Our work with this method has been concerned chiefly with the isolation of anaerobes, especially *B. aerogenes capsulatus*, from stools but has proved equally applicable to material from various other sources. Tubes of milk from which the cream has not been removed are boiled for several minutes to drive off all dissolved

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air. These are then cooled and inoculated with the stool or other material being studied, and heated at 80° C. for 20 minutes. After 24 hours' incubation those tubes which give a characteristic reaction for *B. aerogenes capsulatus* are set aside for "plating."

Dilutions are made from these milk cultures by shaking up several drops, drawn from the bottom of the tube, in sterile water. Bent glass rods dipped in this dilution are then rubbed over the surface of the medium in the bottles, and closely fitting one-holed rubber stoppers carrying a closely fitting glass tube four inches in length are inserted in the mouths of the bottles. The outer end of the tube projects three-fourths of an inch beyond the stopper and carries a tightly fitting rubber tube three inches in length. The inner one inch of the tube is bent at an angle of 45° and the stopper turned so that the end of the tube points toward the side of the bottle opposite the medium.

As much air as possible is aspirated from the bottle and the rubber tube closed with a pinch-cock. The bottle is now placed on its side with medium uppermost and with a pipette 10 c.c. each of a 50 per cent solution of pyrogallic acid and 10 per cent solution of sodium hydroxid are run in through the rubber tube, care being taken to avoid letting air into the bottle. A few cubic centimeters of clean water are allowed to run in, with the same precautions, to free the rubber tube of alkali. Rubber gloves should be worn while introducing the fluids in order to avoid staining the hands. The efficiency of the apparatus and the absence of leaks are indicated by the presence of fluid in the glass tube and by the collapsed condition of the rubber tube behind the pinch-cock. Both these conditions are present if the apparatus is working properly. The bottle must be kept constantly on its side with the medium uppermost.

After incubation for 24 hours, the pinch-cock is carefully opened and the air allowed to flow in very gently to avoid spattering the fluid up on the medium. The stopper is then removed and the fluid poured out. Water is run in carefully and all the black fluid washed out. Care must be taken not to splash any water up on the surface of the medium. The bottles are now stood on end, mouth down, for 15 or 20 minutes to drain. If the litmus-

dextrose-egg medium is used the color will usually return to the litmus while the bottles are draining.

After the bottles have drained, colonies are fished and planted on slants of dextrose blood agar, dextrose agar, or dextrose-egg-medium. These are then inverted, without plugs, in a wide-mouthed jar (a pint fruit jar serves well) containing 5 to 10 gms. of dry pyrogallic acid. When the jar is nearly full of tubes water is poured in to a depth of three-fourths of an inch. As soon as the pyrogallic acid is completely dissolved the solution is covered with a layer of paraffin oil and 20 c.c. of a 10 per cent solution of sodium hydroxid is introduced below the oil by means of a pipette. The tubes are then incubated. This method is essentially that described by Rickards.[1] These slant cultures will usually be found to be pure, and are ready for use after 24 hours' incubation.

The method is simple, easy of application, makes possible the obtaining of pure cultures in the shortest possible time, and has proved very efficient and useful in our hands.

[1] *Centralbl. f. Bakt.*, I Abt., O. 1904, 36, p. 557.